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14. ABSTRACT
Experiments to test pure protein on live human cells in vitro require the removal of all contaminants from protein expression. In order to do so, gel filtration is commonly employed as the highest level of purification. Gel filtration experiments can only be conducted using an appropriate purification machine. With this appropriate equipment, protein has been purified from a yeast expression system to explore the effects of that protein on human lung cells. The purpose of these experiments are to investigate the immune response when cells are stimulated with highly purified chilactin protein. Thus far, the protein has been successfully expressed and purified. The next steps will be

15. SUBJECT TERMS
Protein Purification

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Report Title

Final Report: 2014 Salish Kootenai College Equipment Grant

ABSTRACT

Experiments to test pure protein on live human cells in vitro require the removal of all contaminants from protein expression. In order to do so, gel filtration is commonly employed as the highest level of purification. Gel filtration experiments can only be conducted using an appropriate purification machine. With this appropriate equipment, protein has been purified from a yeast expression system to explore the effects of that protein on human lung cells. The purpose of these experiments are to investigate the immune response when cells are stimulated with highly purified chilectin protein. Thus far, the protein has been successfully expressed and purified. The next steps will be to stimulate the cells and to isolate RNA for RTPCR to identify immune gene expression.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

none

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Christina L. Rush	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Erin LaMere	0.00	Life Sciences B.S.
Amber Hamm	0.00	Life Sciences B.S.
Meg Sherry	0.00	Life Sciences B.S.
FTE Equivalent:	**0.00**	
Total Number:	**3**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

We have successfully purified human YKL39 chilectin protein from a yeast expression system. We are currently working on purification of a human chitinase, AMCase. Summer research has concluded and fall quarter will begin studies of these highly purified protein on human lung cells. It is expected that the two students dedicated to the project will be able to dedicate 5 hours per week per student during the quarter. Work will continue in the winter and spring quarters. Summer 2017 is planned to complete this project and begin writing for a publication. Students can work up to 38 hours per week in the 10 week summer period at which time most of the research at an undergraduate institute is completed. After the summer 2017 period, a new project to purify protein will begin with the idea to teach undergraduate students the techniques of protein purification and subsequent crystallization of a protein for structure determination. This future project is in preliminary stages of development.

Technology Transfer

none

Statement of the problem studied

To study protein stimulation on human cells in vitro, protein must be highly purified after expression. Gel filtration is the method to purify proteins to the highest level and can only be conducted using a protein purification unit. The problem was that we did not have a protein purification unit to conduct high level purification.

Summary of the most important results

Protein was successfully purified over the 2016 summer research period. A total of 15 mg/ml and 3 ml of protein was purified of human YKL39. AMCAse, a human chitinase was also purified via SP purification and will be gel purified over the fall quarter which begins on the 26th of September. Stimulation of human lung cells to investigate the effect of the proteins on immune system genes will be evaluated using RTPCR over the following year.

Bibliography

Recklies, A.D., Ling, H., White, C., and Bernier, S.M. (2005). Inflammatory cytokines induce production of CHI3L1 by articular chondrocytes. J Biol Chem *280*, 41213-41221.

Reece, J.J., Siracusa, M.C., and Scott, A.L. (2006). Innate immune responses to lung-stage helminth infection induce alternatively activated alveolar macrophages. Infect Immun *74*, 4970-4981.

Human **YKL-39** is a pseudo-chitinase with retained chitooligosaccharide-binding properties.

Schimpl M, Rush CL, Betou M, Eggleston IM, Recklies AD, van Aalten DM.

Biochem J. 2012 Aug 15;446(1):149-57.